

What is claimed:

1. An EPO peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.
2. The EPO peptide of claim 1, wherein said one or more glycans is a
5 monoantennary glycan.
3. The EPO peptide of claim 1, wherein said one or more glycans is a biantennary glycan.
4. The EPO peptide of claim 1, wherein said one or more glycans is a triantennary glycan.
- 10 5. The EPO peptide of claim 1, wherein said one or more glycans is at least a triantennary glycan.
6. The EPO peptide of claim 1, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.
7. The EPO peptide of claim 1, wherein said one or more glycans is selected
15 from an N-linked glycan and an O-linked glycan.
8. The EPO peptide of claim 1, wherein said one or more glycans is at least two glycans selected from an N-linked and an O-linked glycan.
9. The EPO peptide of claim 1, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.
- 20 10. The EPO peptide of claim 9, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a fungal cell.
11. The EPO peptide of claim 10, wherein said fungal cell is a yeast cell.
12. A glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan,

wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase.

13. The glycoPEGylated EPO peptide of claim 12, comprising at least one
5 mono-antennary glycan.

14. The glycoPEGylated EPO peptide of claim 12, wherein all of said glycans are N-linked and are mono-antennary.

10 15. The glycoPEGylated EPO peptide of claim 12, wherein all of said glycans are N-linked and at least one of said glycans comprise said poly(ethylene glycol).

16. The glycoPEGylated EPO peptide of claim 15, wherein more than one of said glycans comprises said poly(ethylene glycol).

15 17. The glycoPEGylated EPO peptide of claim 12, wherein all of said glycans are N-linked and all of said glycans comprise said poly(ethylene glycol).

18. The glycoPEGylated EPO peptide of claim 12, comprising at least three
20 mono-antennary glycans having said poly(ethylene glycol) covalently attached thereto.

19. A glycoPEGylated EPO peptide, wherein said EPO peptide comprises three or more glycans.

25 20. The glycoPEGylated EPO peptide of claim 9, wherein at least one of said glycans comprises said poly(ethylene glycol) covalently attached thereto.

21. The glycoPEGylated EPO peptide of claim 18, wherein more than one of said glycans comprises said poly(ethylene glycol) covalently attached thereto.

30

22. The glycoPEGylated EPO peptide of claim 18, wherein all of said glycans comprise said poly(ethylene glycol) covalently attached thereto.

5 23. The glycoPEGylated EPO peptide of claim 12 wherein said poly(ethylene glycol) is linked to at least one sugar moiety selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and a sialic acid (SA).

24. The glycoPEGylated EPO peptide of claim 23, wherein said sialic acid is N-acetylneuraminic acid.

10 25. The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide does not comprise an O-linked glycan.

26. The glycoPEGylated EPO peptide of claim 12 wherein said EPO peptide comprises at least one O-linked glycan.

15 27. The glycoPEGylated EPO peptide of claim 26, wherein said O-linked peptide comprises said poly(ethylene glycol) covalently attached thereto.

28. The glycoPEGylated EPO peptide of claim 27, wherein said EPO peptide
20 is recombinantly expressed in a cell.

29. The glycoPEGylated EPO peptide of claim 28, wherein said cell is selected from the group consisting of an insect cell, a fungal cell and a mammalian cell.

25 30. The glycoPEGylated EPO peptide of claim 29, wherein said fungal cell is a yeast cell.

31. The glycoPEGylated EPO peptide of claim 29, wherein said cell is an insect cell.

30

32. The glycoPEGylated EPO peptide of claim 29, wherein said cell is a yeast cell.

5 33. The glycoPEGylated EPO peptide of claim 29, wherein said cell is a mammalian cell.

34. The glycoPEGylated EPO peptide of claim 33, wherein said mammalian cell is a CHO cell.

10 35. The glycoPEGylated EPO peptide of claim 12, wherein said poly(ethylene glycol) has a molecular weight selected from the group consisting of about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 20 kDa, 30 kDa and 40 kDa.

15 36. The glycoPEGylated EPO peptide of claim 35, wherein said poly(ethylene glycol) has a molecular weight of 20 kDa.

20 37. The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide is selected from the group consisting of a naturally occurring EPO peptide and a mutated EPO peptide.

38. The glycoPEGylated EPO peptide of claim 37, wherein said mutated EPO peptide comprises the amino acid sequence of SEQ ID NO:73 having at least one mutation selected from the group consisting of Arg¹³⁹ to Ala¹³⁹, Arg¹⁴³ to Ala¹⁴³ and Lys¹⁵⁴ to Ala¹⁵⁴.

25 39. A method of making a glycoPEGylated EPO peptide, said method comprising the step of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar covalently linked to poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide.

30

40. The method of claim 39, wherein the sugar of said nucleotide sugar is selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and a sialic acid (SA).

5 41. The method of claim 40, wherein said sialic acid is N-acetylneuraminic acid (NAN).

 42. The method of claim 39, wherein said poly(ethylene glycol) has a molecular weight selected from the group consisting of about 1 kDa, 2 kDa, 5 kDa, 10 kDa,
10 20 kDa, 30 kDa and 40 kDa.

 43. The method of claim 42, wherein said poly(ethylene glycol) has a molecular weight of 20 kDa.

15 44. The method of claim 39, wherein said EPO peptide is recombinantly expressed in a cell.

 45. The method of claim 44, wherein said cell is selected from the group consisting of an insect cell, a fungal cell and a mammalian cell.

20 46. The method of claim 45, wherein said cell is an insect cell.

 47. The method of claim 45, wherein said cell is a yeast cell.

25 48. The method of claim 45, wherein said cell is a mammalian cell.

 49. The method of claim 48, wherein said mammalian cell is a CHO cell.

30 50. The method of claim 39, wherein said EPO peptide is selected from the group consisting of a naturally occurring EPO peptide and a mutated EPO peptide.

51. The method of claim 50, wherein said mature EPO peptide has the sequence of SEQ ID NO:73.

52. The method of claim 50, wherein said mutated EPO peptide comprises the amino acid sequence of SEQ ID NO: 73 having at least one mutation selected from the group consisting of Arg¹³⁹ to Ala¹³⁹, Arg¹⁴³ to Ala¹⁴³ and Lys¹⁵⁴ to Ala¹⁵⁴.

53. The method of claim 39, wherein before step (a):

(b) contacting said EPO peptide with a mixture comprising a nucleotide-N-acetylglucosamine (GlcNAc) molecule and an N-acetylglucosamine transferase (GnT) for which the nucleotide-GlcNAc is a substrate under conditions sufficient to form a bond between said GlcNAc and said EPO, wherein said GnT is selected from the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V and GnT VI.

54. The method of claim 53, wherein said mixture comprises one GnT selected from the group consisting of GnT I, GnT II, GnT IV, GnT V and GnT VI.

55. The method of claim 54, wherein said GnT is GnT I.

56. The method of claim 54, wherein said GnT is GnT II.

57. The method of claim 39, wherein said glycoPEGylated EPO peptide comprises at least one mono-antennary glycan.

58. The method of claim 39, wherein the sugar of said nucleotide sugar is galactose and said glycosyltransferase is galactosyl transferase I (GalT I).

59. The method of claim 53, wherein before step (a) but after step (b):

(c) contacting said EPO peptide with a mixture comprising a nucleotide galactose (Gal) and galactosyl transferase I (GalT I) under conditions sufficient to transfer galactose to said EPO peptide.

60. The method of claim 39, wherein in step (a), the sugar of said nucleotide sugar is sialic acid and said glycosyltransferase is a sialyltransferase.

5 61. The method of claim 60, wherein said sialic acid is N-acetylneuraminic acid (NAN).

62. The method of claim 60, wherein said sialyltransferase is selected from the group consisting of $\alpha(2,3)$ sialyltransferase, $\alpha(2,6)$ sialyltransferase and
10 (2,8)sialyltransferase.

63. A glycoPEGylated EPO peptide made by the method of claim 39.

64. A glycoPEGylated EPO peptide, said EPO peptide comprising the
15 sequence of SEQ ID NO:73.

65. A glycoPEGylated EPO peptide, said EPO peptide comprising the sequence of SEQ ID NO:73 and further comprising a mutation in said sequence.

20 66. A method of making a glycoPEGylated EPO peptide, said method comprising the steps of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar covalently linked to poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide, wherein said
25 glycosyltransferase is a fucosyltransferase.

67. The method of claim 66, wherein said fucosyltransferase is selected from the group consisting of fucosyltransferase I, fucosyltransferase III, fucosyltransferase IV, fucosyltransferase V, fucosyltransferase VI and fucosyltransferase VII.

30

68. A glycoPEGylated EPO peptide made by the method of claim 66.

69. The method of claim 66, wherein said EPO peptide is expressed in a CHO cell.

5 70. A method of treating a mammal having anemia, said method comprising administering to said mammal an EPO peptide having one or more glycans having a glycoconjugate molecule attached to said peptide, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal.

10 71. The method of claim 70, wherein said mammal is a human.

72. A method of providing erythropoietin therapy to a mammal, said method comprising administering an effective amount of a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule
15 covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal.

73. The method of claim 72, wherein said mammal is a human.

20 74. A method of treating a mammal having anemia, said method comprising administering to said mammal a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using
25 a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal..

75. The method of claim 74, wherein said mammal is a human.

30

76. The method of claim 75, wherein said anemia is associated with chemotherapy.

77. A method of treating a kidney dialysis patient, said method comprising
5 administering to said patient a glycoPEGylated EPO peptide comprising an EPO peptide and
at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said
glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a
glycosyltransferase, wherein said EPO peptide is administered in an amount effective to
increase the hematocrit level in said patient.

10